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This study aims to determine common features of the response to butyrate, an inhibitor of histone deacetylase, by prostate cancer cell lines. One issue of prime importance is the range of cell phenotypes of susceptible and resistant cell lines. Three out of five poorly differentiated prostate cancer cell lines were nearly fully sensitive to terminal loss of replicative abilities after 48 hr exposure, conditions that are well tolerated by most normal cell types. Addressing our principal hypothesis, that the prototypical butyrate-hypersensitive cell will be undifferentiated and mutant in p53, we will select cell lines that are tolerant of butyrate, determine their p53 functional status (transcriptional response to ionizing radiation) and, if wild type, knock out their p53 protein using an appropriate oncoprotein. We expect that loss of p53 function will confer butyrate hypersensitivity. The work will also address the responsiveness to mezerein, an activator of protein kinase C that increases the proportion of butyrate-sensitive cells that undergo apoptosis in a 24 hr exposure. This agent is an inducer of terminal differentiation in its own right, affecting moderately differentiated adenocarcinoma cell lines. Because such lines are rare among prostate cancer cell lines (in contrast to their abundance as human cancers), this promising agent cannot be adequately studied at the present time.

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John (Jack) McBain Ph.D.

Annual report 1999
Butyrate-Induced Apoptosis in Prostate Cancer Cells
John A. McBain, Ph.D.

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Introduction

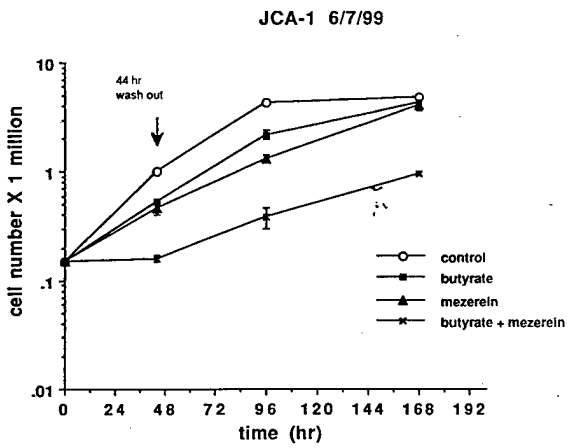
Agents for targeting endogenous mechanisms of differentiation or programmed cell death have shown considerable promise against cell lines from human adenocarcinomas. In certain colorectal cancer cell lines, treatment with butyrate, an inhibitor of nucleosomal core histone deacetylase, can cause apoptotic death of up to half of the cell population. Combinations of butyrate with PKC activators of the mezerein class, can lead to apoptosis of the entire cell population. This is particularly remarkable because of the low toxicity of butyrate against other cell types, either alone or in combination with a PKC activator. The paucity of active agents for treatment of recurrent, hormone-refractory prostate cancer makes such new approaches particularly attractive, and likely to go to clinical trial should cell culture work demonstrate significant promise. This project aims to 1) assess the activity of butyrate on human prostate cancer cells in various culture environments, and 2) identify determinants or markers of susceptibility to apoptosis, differentiation and senescence among prostate cancer cells differing in butyrate responsiveness.

Body

Butyrate-responsiveness of prostate cancer cell lines

Published work on prostate cancer cell line response to butyrate is sparse (1, 2), presenting the sensitivity of PC-3 cells to butyrate, but without details as to the form, rate or extent of the response. (We have demonstrated a <5% survival after a 48 hr exposure). An assessment of the potential of HDA inhibitors as therapeutics for prostate cancer will require testing of as many isolates of prostate cancer as possible. We hypothesized that prostate cancers would be as heterogeneous in sensitivity to butyrate-induced apoptosis as were colorectal cancer cell lines. In the later series of cell lines, butyrate-hypersensitive lines were typically poorly-differentiated (as defined by a lack of expression of proteins typical of the mature, functional cell of a given tissue). Among a small group of colorectal cancer cell lines that expressed moderate amounts of CEA (a marker of glandular differentiation of gastrointestinal and breast epithelium *in vitro* and *in vivo*) none were notably susceptible to butyrate-induced apoptosis, whereas 2 of 4 poorly differentiated colorectal cancer cell lines were found to be hypersensitive to butyrate. In addition, apoptosis-susceptible cells were all mutant or null for p53 protein, a correlation that is superficially opposite to that of susceptibility to genotoxic agents. Cancer-derived cell lines and cultured normal cells that have been examined to date cease growth while incubated with butyrate at concentrations above 3 mM (except for JCA-1, see below). "Hypersensitive" cell types not only cease replicating, but also die in large numbers when cultured in the presence of butyrate. Cell types which are notably prone to butyrate-induced apoptosis are typically derived from cancers, but apparently also present as a fraction of certain rapidly-dividing normal epithelial and hematopoietic cell populations. One of the great challenges of working with HDA inhibitors is thus in defining the range of susceptible cell types, and the environments conducive to loss of replicative abilities. Performance of such work on cultured cells is particularly important in this instance, because experience with this class of agents *in vivo* is limited by pharmacological problems with both butyrate and the available fungal HDA inhibitors trichostatin and trapoxin (M. Yoshida, pers. communication).

Based upon published characterizations of PSA and p53 expression by existing prostate cancer cell lines, we expected to find that at least 3 of the 5 existing poorly-differentiated prostate cancer cell lines would exhibit irreversible growth arrest when cultured in butyrate-containing medium. Characteristics that would justify labeling prostate cancer cell lines as poorly differentiated include androgen-independence, low or no expression of PSA or acid phosphatase, expression of vimentin in addition to cytokeratins, little apical/basal polarization whether in culture or xenograft, and little membrane specialization such as epithelial adhesion complexes. While such cell lines are often considered poorly representative of human prostate cancer for lacking PSA expression and architectural features typical of most prostate cancers, they are likely to represent a subset of the high Gleason-grade cancers that are notable for their aggressive growth *in vivo*, and of the highly progressed cancers that recur as a signal of the inevitable failure of androgen deprivation therapy.



JCA-1 6/28/99

treatment	Plating efficiency	Colony # (Mean +SD)
untreated	.68	68 ± 10
butyrate	.43	43 ± 15
mezerein	.35	35 ± 7
mezerein + butyrate	.00	0 ± 0

JCA-1 7/13/99

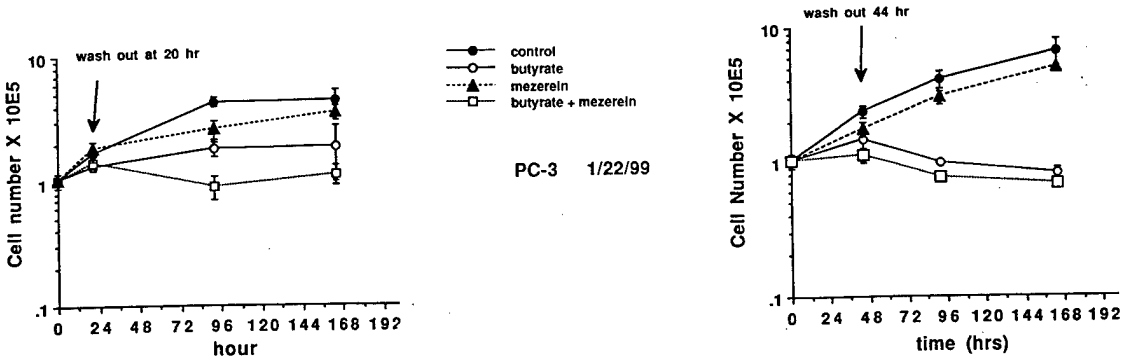
Colony #_+ SD (PE)

# Cells plated	Untreated	Butyrate	Mezerein	Mezerein + Butyrate
100	56±9 (.56)	37±6 (.37)	21±7 (.21)	0±0
300	163 ±11 (.54)	85 ±6 (.28)	57 ±6 (.19)	0±0
1,000	TMTC	268±18 (.27)	231±43 (.23)	1±1
3,000	TMTC	TMTC	TMTC	4±2 *
10,000	TMTC	TMTC	TMTC	1±1 *

* alot of scattered cells

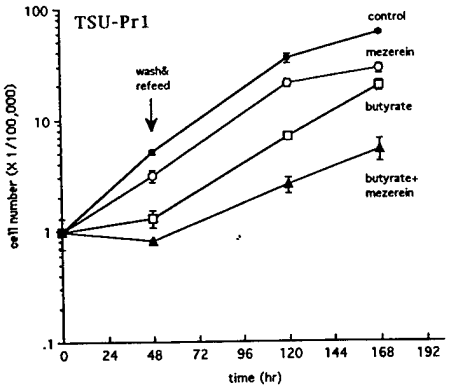
At one extreme of butyrate sensitivity, the JCA-1 line retains over half of its high untreated CFE (0.55-0.70) when cultured in 5 mM butyrate for 48 hr, effects that have proven independent of seeding density. Furthermore, the cell line is the only example in our experience (and survey of the literature) of a cell that can grow in the continuous presence of 5 mM butyrate. This is not likely due to rapid metabolism, because the response is independent of input cell number, although this needs to be formally examined. We have also not yet determined whether or not the cells respond to butyrate with histone hyperacetylation, but the fact that the combination of butyrate plus mezerein is active by several criteria, while mezerein is itself minimally active suggests that the resistance is due to a factor beyond the histone hyperacetylation level.

Of the other poorly-differentiated lines, PC3, DU145, ND-1 and TSU-Pr1, only the later appears capable of survival at a high level after butyrate exposure (and even it succumbs when challenged at low plating densities - see below). For DU145, a period of exposure between 20 and 44 hr is required to effect the maximal reduction of survival. A similar exposure of PC-3 cells leads to a net loss of cells over the succeeding week.



In contrast, when TSU-Pr1 is exposed for 48 hr to 5 mM butyrate in a mass culture (100,000 cells per ml), we estimate that at least 90% of the cells survive (we will need to formally quantitate this response in additional, modified survival assays. We can however conclude that PC-3, DU145 and ND-1 are each very sensitive to butyrate, responding to 48 hr of treatment with 5 mM butyrate with either little subsequent growth or overall reductions (PC-3) of surviving cell numbers.

In what is a recurring theme among prostate cancer cell lines, survival is more dramatically depressed when measured by Colony Forming Efficiency (CFE) assay. The results presented are for experiments performed with cells seeded at 100-10,000 cells per dish rather than 100,000, as used to initiate cultures for 'growth curve' assay, but handled similarly otherwise. For PC-3, DU145 and ND-1 the results are consistent with the growth curve, assuming that cells do not resume growth after butyrate removal and that the apparent slight increase in cell numbers are terminal cell divisions. We do need to test this more formally. However, the results for TSU-Pr1 suggest that there is a clear discrepancy between survival in growth curve assay densities and those at the lower limits of CFE assays. Although the endpoints for the two assays differ by weeks, amounting to 1-2 generations rather than 5-10 generations (a colony of 32 cells represents 5 idealized doublings), it is harder to rationalize the finding that cell numbers increase by 15 fold within 5 days from 100,000 cells, but that the surviving fraction falls 97% of control CFE when cells are treated at a density of 1000 cells per dish. Rather, there appears to be a protective factor present in high density cultures (soluble in medium, present in insoluble 'matrix', or other) as far as tolerance of butyrate-induced growth inhibition is concerned. We haven't formally determined that TSU-Pr1 cells which replicate after exposure to butyrate as mass cultures are competent at colony formation after isolation from one another, nor that cells which fail to form colonies cannot do so when provided with medium 'conditioned' by growth of large numbers of TSU-Pr1 cells. A series of investigations addressing these possibilities are planned.



TSU-pr 11/8/99

			Colony # (PE)			
Cells plated	untreated		butyrate	mezerein		mezerein + butyrate
100	28 ± 4	(.28)	0 ± 0	1 ± 1		0 ± 0
300	64 + 4	(.21)	0 + 1	5 + 1	(.016)	0 + 0
1000	240 + 14	(.24)	3 + 2**	15 + 2	(.015)	0 + 0
3000	TMTC		26 + 5**	109 + 8	(.036)	0 + 0
10,000	TMTC		*	*		5 + 1**
30,000	TMTC		TMTC	TMTC		lawn of cells

* colonies are all clustered together in ring around plate-incubator movement problem

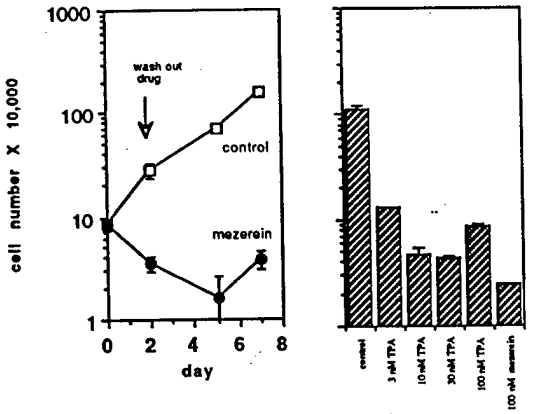
** alot of scattered cells

We have yet to account for the relative contribution of each of the known mechanisms of cell loss, but apoptotic death accounts for only a portion of the inhibition. As part of our investigation of the mechanisms of irreversible growth arrest in each of the three fully-responsive cell lines we will investigate the fate of exposed cells. Cells will be transiently-treated with butyrate in mass cultures, and then examined over the course of subsequent culture in different environments and cell densities to determine whether they suffer a delayed apoptosis or prolonged senescence, or rather changes consistent with differentiation (high adhesion and altered expression of proteins typical of mature epithelial, glandular or prostatic cells).

Mezerein responsiveness of prostate cancer cell lines

Although the prime goals of this project relate to butyrate sensitivity, the use of mezerein as a modulator of butyrate response (see below) has led to several unexpected spinoffs regarding the sensitivity of existing cell lines to activators of protein kinase C. Although we have included these tangential findings here, we will pursue them further only if we can obtain sufficient grant support. Because TSU-Pr1 cells are substantially depressed in CFE by butyrate, and especially by the combination of butyrate and mezerein, this issue will need to be addressed to the level of identifying the factor responsible for protection from mezerein-induced inhibition in high density culture (see below). We hypothesize that the responsible factor is at least partially distinct from that responsible for protection from butyrate-induced growth inhibition.

1) LNCaP, the one line that is known to be sensitive to TPA-induced growth arrest (3), has proven to be even more sensitive to mezerein as a growth inhibitor. Because of the ability of high concentrations of TPA to oppose the antireplicative actions of low doses of TPA (accounting for the frequent findings of biphasic dose response curves for TPA and, more so, for bryostatin 1 - (4)), the optimum concentration of TPA is often near 10 nM (5). On the other hand, mezerein is rarely found to stimulate growth, and so concentrations of mezerein near 100 nM are often more effective than 10 or 100 nM TPA.



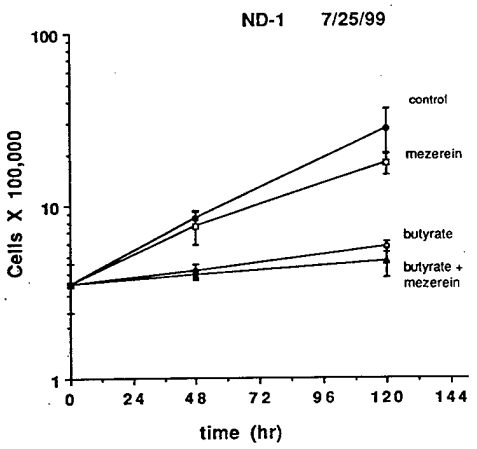
. . . Effects of Mezerein /TPA on LNCaPs
Cells were seeded into 10cm^2 TC dishes. Day 0 (2 days after seeding) Mezerein or TPA was added. After 48 hrs cells were washed (non-adherent cells were retained), refed and incubated in fresh media. Cell counts were done in triplicate.
3a Mean Cell # ± SD. 3b. counts on day 5

2) The other prostate cancer cell lines that have been reported to be affected by TPA, namely TSU-Pr1 and JCA-1, were said to be altered in differentiation, largely on morphological grounds (6, 7) (our findings as well). Although it was not detailed in the earlier publications, we have found them to be almost unaffected in growth rate when treated in mass culture.

However TSU-Pr1 exhibits dramatically decreased colony forming ability when treated with mezerein at low cell densities.

3) In addition to JCA-1, both PC-3 and DU-145 are essentially resistant to mezerein-induced growth arrest, even when plated at low cell density. Because the mezerein-treated cultures respond in a morphological sense, and exhibit synergistic inhibition of survival when treated with HDA inhibitors, these cells serve as mezerein-resistant controls for experiments focussing on mezerein-induced depression of survival.

4) Both ND-1 and TSU-Pr1 (see above) are only minimally growth inhibited by mezerein in high density cultures with cell densities typical of growth curves, but are inhibited by mezerein in sparse culture. But both of these lines are highly inhibited in colony forming ability after treatment with mezerein in cultures of low cell density, but have appreciable CFE when exposed to mezerein at densities near 300 cells per cm^2 (3000 per dish).



7/13/99 ND-1 Colony Forming

X ± SD (% PE)

cells plated	control	butyrate	mezerein	mezerein + butyrate
100	22 ±10 (22)	3 ± 2 (3) *	1 ± 1 (1) *	0 + 0 (0)
300	62 ± 4 (21)	8 ± 4 (3)	1 ± 1 (0.3)	0 + 0 (0)
1000	206 ±15 (21)	7 ± 2 (0.7)	5 ± 3 (0.5)	0 + 0 (0)
3000	TMTC	19 ± 5 (0.6)	7 ± 2 (0.2)	0 + 0 (0)
10000	TMTC	61 ± 17 (0.6)	344 ± 41 (3)	0.3 + 0.6 (.003)
30000	TMTC	TMTC	TMTC	0 + 0 (0)

* alot of scattered cells and small colonies less than 50 cells each are visible on butyrate or mezerein treated plates. Not seen on combined Butyrate + mezerein Rx

Encouragingly, colony forming abilities are quite similar regardless of seeding density for untreated cells, suggesting that any crossfeeding or autocrine stimulation is not limiting for normal growth, but only for survival during an alternative mezerein-induced physiological state (neoplasia, metaplasia or differentiation). We consider this phenomenon of potential importance to the basic mechanism which limits establishment of prostate cancers as cell lines, because terminal differentiation is one of the principal means by which explanted epithelia die out in culture. The relationship between mezerein-induced inhibition of ND-1 and TSU-Pr1 CFE and the inhibition of LN-CaP growth is unknown, but should be made clearer by identifying conditions for alleviating one or the other inhibitory actions.

In trying to determine the factors present in high cell density cultures, we are pursuing both diffusible growth factor and matrix elements, and entertaining the possibility that mezerein may induce expression of protective or inhibitory factors. In order to determine the impact of cell density on apparent mezerein responsiveness, we will be trying to separate the effects of 'conditioning' of the culture medium and dish (matrix) by culture before mezerein treatment and the effects of substituting conditioned medium for fresh medium added at the start of the recovery / growth phase. We have already found several defined extracellular matrices to have no appreciable effect on sensitivity to mezerein. However, the possibility that critical factors present in conditioned medium might be required as a component of such a substitute matrix

cannot be ruled out. Because of our inclination to think of mezerein-induced growth arrest as a terminal differentiation, we will be examining treated cells for features of this form of cell loss, (expression of maturation-associated proteins, shedding of cell surface HSPG, secretion of polyamines, or formation of a cross-linked submembranous envelope), as well as the competing mechanisms of apoptosis (caspase-mediated specific protein cleavage) and senescence (specific galactosyltransferase).

Response to the combination of butyrate and mezerein

Activators of protein kinase C, such as TPA and mezerein, synergize with butyrate in several colorectal cancer cell lines that already exhibit a moderate incidence of butyrate-induced apoptosis (5). These effects seem to include increases in both incidence and rate of apoptosis induction. Our work with colorectal cancer cell lines has focussed on the synergistic depression of survival in cells treated with butyrate and TPA. Because bryostatin, an activator of PKC with predominantly proreplicative activities, was inefficient in promoting butyrate-induced apoptosis (actually blocking the apoptosis-promoting effects of TPA), and because TPA itself can exhibit dose response characteristics which are a composite of antireplicative and proreplicative actions, we have more recently used mezerein to probe the potential synergy of PKC activation with histone deacetylase inhibition. Mezerein (and teleocidin) are PKC activators with even less proreplicative activity than TPA.

In each prostate cancer cell line that we have examined thus far, including JCA-1 which is largely resistant to either of these agents alone, the combination of butyrate and mezerein leads to a major reduction of colony forming ability (see above). As with the effects of butyrate itself, the combination is considerably less active when examined in mass cultures. Experiments where the cell seeding density was varied indicated an expectedly higher proportional survival when cells were seeded at high density, as expected from the results of experiments with either butyrate or mezerein themselves. Such cell density dependence was much less evident among colorectal cancer cell lines, perhaps because autocrine growth stimulation evolved to such a high level in prostate cancers, and even more so in androgen-independent cancers and their derived cell lines.

Determinants of susceptibility to butyrate-induced growth arrest, and mechanisms of resistance by JCA-1 and high density TSU-Pr1 cell cultures

It is somewhat difficult to state at this moment whether the responses of the JCA-1 cell line to butyrate (growth in the continual presence) and to butyrate and mezerein (complete growth inhibition at clonal densities) are as unusual as they at first appear. Only in one published example, of a liver-derived cell line, was a mammalian cell shown to be capable of growth in millimolar concentrations of butyrate, and that cell had been selected for resistance to growth in butyrate. We set out to find a cell line that could resist butyrate- and butyrate+mezerein-induced apoptosis similar to that of the HCT116 cell line. Clearly, JCA-1 has tolerance to the survival- and growth-inhibiting actions of butyrate. We concede that we have in the past rarely put as much effort into studying the actions of the combination of butyrate and TPA (or mezerein) on cells plated at clonal densities. The continued investigation of these cells may show them to resist butyrate in a manner similar to HCT116 and/or normal epithelia. Alternatively, we may find that the cells are a fortuitous artifacts of nature which allow us to identify a unique mechanism of resistance. Conversion of JCA-1 from resistant to hypersensitive to butyrate-induced apoptosis (see below) may help us determine why such a large proportion of existing prostate cancer cell lines are butyrate hypersensitive, and point

out the determinant that can be assayed in clinical cancers to assess sensitivity to HDA inhibitor treatment. The mechanisms of both the sensitivity to butyrate-induced growth arrest and the resistance conferred by high cell density remain priorities for continued investigation.

The planned investigation of p53 function in the tolerance of butyrate by JCA-1 will include the genetic and cell biological experiments outlined in the original proposal and statement of work. The abundance of p53 will be reassessed, and the functional competency will be evaluated as planned. Nonetheless, we can borrow from published studies the following presumptive genotypes. The LN-CaP cell line has been found to be wild type p53 in sequence and expression , while the JCA-1 line resembles LNCaP in expressing only a moderate level of p53 protein, consistent with its carrying wild type genes. The PC3 and TSU-Pr1 lines are in contrast considered null in p53 expression, while both ND-1 and DU145 contain abundant p53 protein, including species presumed to be derived from a mutant allele. We are thus inclined to use JCA-1 as a p53 wild type, butyrate-tolerant line, for our investigation of the effects of E6 oncoprotein-directed p53 knockout. This is predicted to increase sensitivity to butyrate-induced death, and contribute to the specific strategy for analysis. We need to proceed with our analysis of p53 functionality. It is interesting that JCA-1 has been so neglected by the general scientific and prostate cancer research community. In spite of the likelihood that it is far from representative of the average prostate cancer cell, it phenotypic resemblance to TSU-Pr1 (D. Groveman, personal observation) suggests that it is not fully unique, and may represent a fully progressed prostate cancer cell type.

Cell line establishment

When we began this study, we predicted that we would find at least one cell line among existing lines that would be highly sensitive to butyrate-induced terminal growth arrest (as mentioned above, we have 3 or 4), and one or more poorly differentiated lines with high resistance (the JCA-1 is at least that). But we thought it likely that we would find a response to butyrate so peculiar that we would want to determine the frequency of such a phenotype within the larger set of prostate cancers. Cell lines are of course the most accessible model system for rapid assessments of this sort. As with all cell line-intensive investigations, one of the strongest indicators of relevance to sporadic cancers of a phenomenon discovered in one cell line is its replication in another line of independent origin. For our study of phorbol ester-induced terminal differentiation of the VACO 10M cell line, the finding of sensitivity in the line derived from the primary tumor (VACO 10P) was evidence of the phenomenon not being due to some chance mutation and evolution in culture, but the finding of high sensitivity in the VACO 1 line attested to its general relevance. However, the best acceptance of its relevance will likely come with my eventual publication of the replication of the phenomenon in 5 of 11 colorectal cancer cell lines of wider circulation. However this type of survey is only valuable when the full range of variation among the clinical cancer is represented among available cell lines. Such is not the case with prostate cancer.

It is well recognized that cell line establishment from primary prostate cancer is a rare event. Nonetheless, no one has presented data that would allow assessment of the actual frequency, or ventured a guess as to an indication of features, molecular or pathophysiological, that might increase the chances of success at prostate cancer cell line establishment. We hypothesized that only those prostate cancers capable of metastasis would yield cell lines. This inference was based upon our work with colorectal cancer cell line establishment (8), and published accounts of prostate cancer cell line establishment. A sizable fraction (est. 25-50%) of all prostate

cancers yield metastases in spite of a lack of evidence of spread at the time of resection, in other words, many cases of metastatic prostate cancer occur in men who have had prostatectomies.

For prostate cancer, between 7 and 10 prostate cancer cell lines with unique origins exist. As one might suspect, at least one instance of cross-contamination between an established cell line and a culture of a prospective cell line has been documented (PPC-1 = PC-3, A. Brothman, personal communication). We estimate the total number of attempts to be about 300-500, based upon discussions with established prostate cancer researchers and those who have had success in establishing cell lines. The vast majority of these were primary cancers, and the typical surgical service is unwilling to cut into or give away any lymph node material, because of the potential for compromising clinical care should the possible tumor-containing portion be lost to the research lab.

Contrary to my original prejudice, I now doubt that this low success rate can be improved by diligent techniques applied to surgical excess primary prostate cancer tissue. I had predicted that carefully retaining all cell progeny, and delaying passage of epithelia as originally projected would allow cancer cells to survive and accumulate. Because prostatectomy is rarely performed when evidence of nodal or distant spread is present, and the growth of metastasis-capable prostate cancer appears to be better in extraprostatic sites, such as bone, one would expect that a resected prostate cancer with metastasis-competent cells might seldom be available to the lab. Alternatively, the hypothetical growth-suppressive actions of prostate tissue might be replicated in tissue culture. Out of approximately 40 prostatectomy-derived cancers, we have had not a single culture survive for more than 3-4 months, corresponding to the time it takes for the prostate-derived fibroblasts to senesce. The epithelial cell outgrowth was typically vigorous, and grew/migrated centripetally, together with presumptive fibroblasts, from small explants of minced tissue. Such explant culture was better than from collagenase-disaggregated tissue, although both methods were used for most tumors. Attempts to pass fractionated epithelial cells, using differential detachment, attachment, enzymatic dissociation or centrifugation, or growth on defined extracellular matrices or in conditioned medium derived from PC-3 prostate cancer cell cultures failed to provide subpopulations differing from normal tissue-derived epithelia. We presume that neoplastic epithelia were often present in these primary cultures, and may have persisted among the epithelia that grew readily out of these explants and tissue dissociates (with or without enrichment for aggregates). But any neoplastic cells must have exhibited a behavior that is indistinguishable from the range of phenotypes characteristic of normal epithelia, because we generally set up parallel cultures from the contralateral normal region of the resected prostate gland. In short, early cancer cells appear to behave in a similar fashion to normal prostatic epithelium, or else are lost rapidly after tissue dissociation or culture (9). For example, growth of the cancer cells may be inhibited by products of normal prostate cells that grow out and/or differentiate upon culture. Because of the paucity of good markers of neoplasia these issues are difficult to resolve.

As mentioned below, we are in the process of applying for grant support to culture (and xenograft) bone marrow biopsy tissue from persons with advanced (bone scan positive, hormone-refractory) prostate cancer, and from autopsy-derived bone tissue. We predict that these cancers would be far more competent at cell culture and transplantation, but will go further and culture bone stromal cells to use as established feeder cells to culture bone stroma-dependent cancer cells, and to identify the factors in bone (predicted to be among the extracellular matrix and associated growth factors).

Butyrate-responsiveness of primary prostate cancer-derived cultures

We have made use of the pairs of cancer-derived and the corresponding normal tissue cultures to ask whether such epithelia differ in survival or morphological response to butyrate and mezerein. Because of the presence of fibroblasts, presumptive basal prostatic epithelia, and the likely neoplastic cells in cancer-derived cultures, such assays are little more than qualitative. Nevertheless, we find that normal and cancer-derived cultures are quite similar in responsiveness, with the major response being an induction of vacuolization in the epithelia of cultures treated with either agent. Because this occurs progressively in an increasing number of epithelial cells as a matter of course in such cultures, we believe that this represents the acceleration of a differentiation that is otherwise spontaneous when the environment is suboptimal, and have not pursued these phenomena further. However, it is remarkable that in none of the 5 culture pairs we tested in this way was a preferential survival or death of cancer-derived cells apparent. We have used both serial imaging with the digital microscope, and solubilization of the entire cell population for measurement of apoptotic proteolysis or gene expression typical of differentiation. Because of uncertainty as to the nature of the epithelial cells, little credence has been paid to these results.

Conclusions

Existing prostate cancer cell lines are heterogeneous in their response to butyrate and mezerein. The LNCaP line is the only one that responds to mezerein with growth arrest, as expected from our experience with colorectal and breast cancer cell lines, in which only moderately-differentiated lines could be induced to undergo terminal differentiation as a response to phorbol esters. PC-3, ND-1, DU145, and TSU-Pr1 are hypersensitive to butyrate, with the first three having the smallest surviving fraction. The JCA-1 cell line appears able to grow in the continuous presence of butyrate, an unprecedented finding which will aid our investigation of the determinants of butyrate sensitivity. All cells exhibit at least an additive response to the combination of butyrate and mezerein. Sparse cultures are clearly more sensitive to growth inhibitory effects of these agents.

Plans

1) Grant funding of a study of prostate cancer cell survival and response to terminal differentiation inducers when cultured in bone-like microenvironment. Establishment of prostate cancer cell lines from marrow of persons with advanced (bone scan positive) cancer of prostate (and breast). We have applied for research support to culture and characterize bone-resident prostate cancer cells, and to establish model systems for characterizing the prostate cancer cells responses to the bone microenvironment. The hypothesis for design of mechanistic experiments has been that fibrillar collagen and non-collagenous bone proteins promote the stabilization of certain key components of the tumor cells' endogenous basal lamina, and thereby an improved response to bone localized growth factors and sialoproteins. The proposal has been sent to PhARMA foundation, American Cancer Society, CaP-CURE and will be submitted to the Veteran's Administration Medical Research Service for Merit Review consideration before 21 D

2) Publication of butyrate and mezerein responsiveness of PrC cell lines.

3) Continued work on p53/nB sensitivity mechanistic hypothesis.

4) Characterization of PrC cell lines - quantitative measures of epithelial/mesenchymal transition, prostatic differentiation, lineage fidelity and mezerein/TPA/bryostatin responsiveness (comparing multiple endpoints for survival).

Abbreviations

PKC	Protein kinase C
TPA	a phorbol ester, activator of PKC
HDA	histone deacetylase
CEA	a cell membrane protein typical of glandular epithelia
PC-3	prostate cancer cell line
ND-1	prostate cancer cell line
TSU-Pr1	prostate cancer cell line
JCA-1	prostate cancer cell line
LN-CaP	prostate cancer cell line
DU-145	prostate cancer cell line
CFE	Colony forming efficiency
CFU	colony forming unit, either a single cell or a cluster of 2-4 cells present at the time of experimental treatment that will be challenged to survive and grow into a colony.

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